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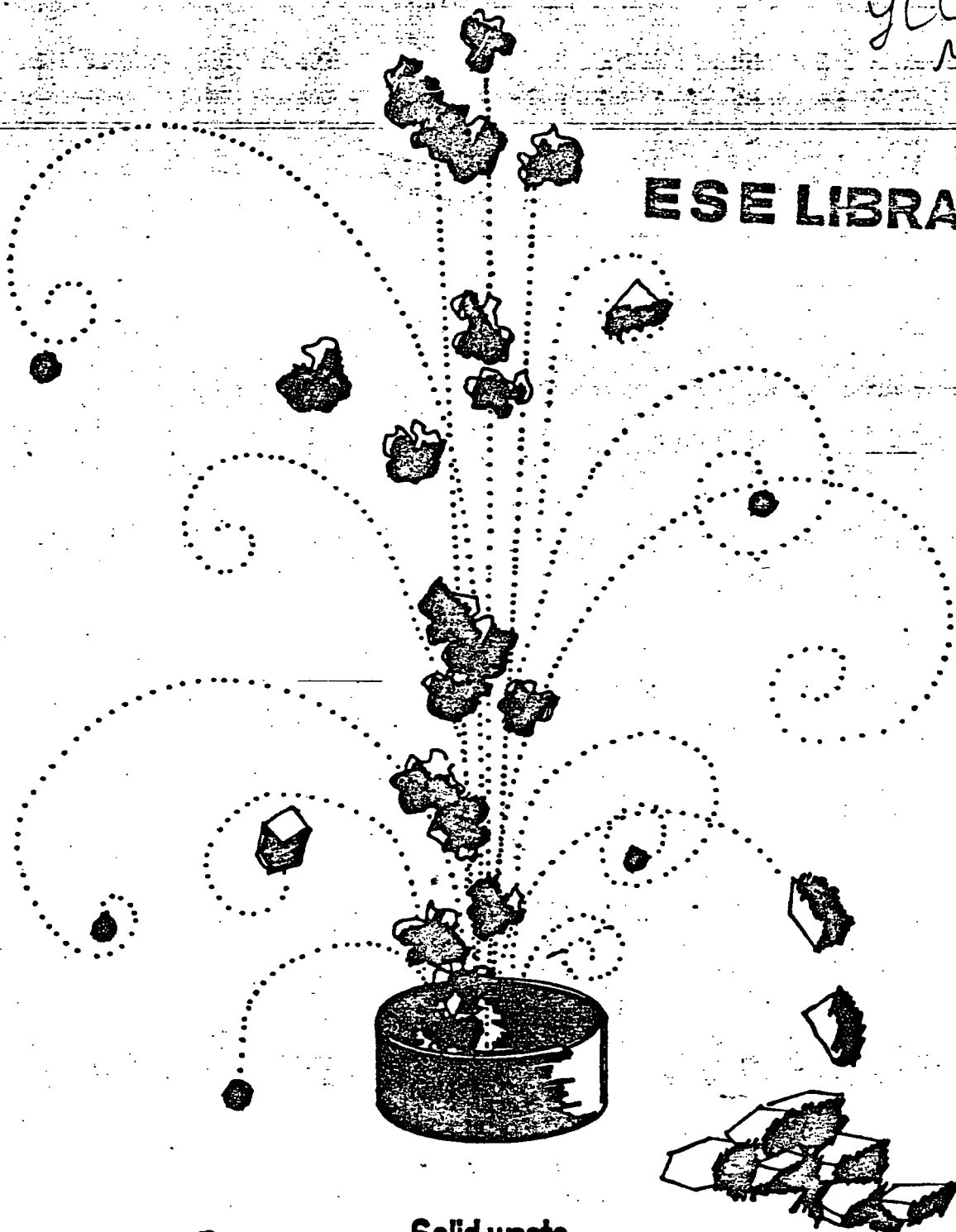
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T = reaction temperature, °K
 w = observed weight gain, g/g of solid⁻¹
 W_{Al} = maximum weight gain corresponding to the stoichiometric sulfation of alumina, g/g of solid⁻¹
 W_K = maximum weight gain corresponding to the stoichiometric sulfation of potassium oxide, g/g of solid⁻¹

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Occurrence of Hexachlorophene and Pentachlorophenol in Sewage and Water

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■ Concentrations of hexachlorophene (HCP) and pentachlorophenol (PCP) in sewage and water samples have been analyzed by gas chromatography. HCP and PCP levels in 24-hr composite samples of sewage influent collected simultaneously from three Oregon cities ranged between 20-31 ppb and 1-5 ppb, respectively. Composite effluent values from these same sewage treatment plants were 6-12 ppb HCP and 1-4 ppb PCP, reflecting a 60-70% removal of HCP and a 4-28% removal of PCP. Analyses of daily and hourly water samples from the Willamette River collected just upstream from the city of Corvallis, Ore., showed that HCP and PCP were present in river water in concentrations varying between 0.01-0.1 ppb and 0.10-0.70 ppb, respectively. Conventional processing of raw Willamette River water at the Corvallis Taylor water treatment plant removed about 60% of the HCP and PCP originally present in the water leaving about 40% of these chlorophenols in the finished drinking water. Identifications of HCP and PCP in sewage effluent and influent, Willamette River water and treated drinking water were confirmed by mass spectrometry.

Increasing amounts of domestic and industrial waste are being discharged into surface waters. Some of these wastes contain toxic chemicals such as chlorophenols, which resist degradation and tend to persist in the environment where they may create serious public health and water quality problems.

Hexachlorophene [HCP, 2,2'-methylene-bis(3,4,6-trichlorophenol)] and pentachlorophenol (PCP) are two such chlorophenols, widely used in our society in a variety of fungicidal or germicidal applications. HCP is employed primarily in consumer products including soaps, cosmet-

ics, and germicidal preparations, whereas PCP is used extensively in industry as a preservative and for slime control. PCP has been found in streams and accumulated in fish tissues following industrial discharge (Stark, 1969; Rudling, 1970), and HCP is thought to persist similarly in surface waters for long periods (Bandt and Nehring, 1962).

Because HCP and PCP are highly toxic chemicals (Nakae et al., 1973; Deichmann et al., 1942), we have made use of a sensitive gas chromatographic method to determine the levels of HCP and PCP in municipal sewage from three Oregon cities (Corvallis, Eugene, and Salem); in the Willamette River that receives the sewage discharge from the three plants; and in a public water supply that employs treated Willamette River water. The presence of HCP and PCP in sewage and in raw and treated river water has also been confirmed by mass spectrographic analysis.

Materials and Methods

Chemicals. Hexachlorophene (U.S.P. grade) was a gift of the Givaudan Corp., Clifton, N.J., and was twice recrystallized from isopropanol-water to yield a purified product with mp at 165-6°C. Reagent grade pentachlorophenol was purchased from Eastman Organic Chemicals, Rochester, N.Y. All solvents or chemicals were reagent grade obtained from commercial sources and were further purified as necessary. Ethereal diazomethane (0.5 mmol/ml) was prepared from Diazald (Aldrich Chemical Co., Milwaukee, Wis.) according to the directions provided by the manufacturer and stored at -4°C until used. Methylene-¹⁴C-hexachlorophene with a specific activity of 2.77 mCi/mmol was obtained from New England Nuclear Corp., Boston, Mass.

Apparatus. Gas chromatography (gc) was carried out in a Varian Aerograph Hy-Fi 600D gas chromatograph equipped with a tritium foil electron capture detector.

Mass spectrometry was performed with a Varian Atlas CH-7 mass spectrometer. A majority of the samples were introduced into the instrument via the inlet probe. In

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some cases, however, crude extracts were injected directly into a Varian Model 1400 gas chromatograph coupled to the mass spectrometer.

Radioactivity was measured by liquid scintillation counting in a Packard Model 314-EX liquid scintillation counter (Packard Instrument Co., Downers Grove, Ill.).

Preparation of Extracts. At neutral pH, small amounts of HCP can be absorbed from aqueous solutions onto glass surfaces, presumably because of the strong tendency of HCP for chelation (Adams, 1958). Such binding to glass could cause errors in analyzing low concentrations of HCP, particularly in solutions containing little silt or organic matter. Therefore, to help saturate any such HCP binding sites, raw Willamette River water and treated water samples were collected from the Corvallis Taylor water treatment plant in 2-liter Pyrex reagent bottles after thorough prerinse of the bottles with the water being sampled. The water samples were also analyzed within 48 hr of collection to minimize any additional losses of HCP. Because of the presence of organic matter and the higher HCP concentrations involved, adsorption to the walls of the containers did not present a problem in the case of sewage samples. Sewage was, therefore, collected directly without prerinse in wide-mouthed jars. To help reduce the possibility of HCP contamination, all glassware used in this study was also subjected to a basic methanol wash step as part of the normal dishwashing procedure.

Sewage influent or effluent (100 ml) or 900 ml of river water were acidified with 4N H_2SO_4 and extracted 3 times with 40-ml portions of $CHCl_3$. Sewage samples and an occasional water sample containing material interfering with the gc analysis were also subjected to a basic wash step. The $CHCl_3$ extracts from such samples were washed twice with 1N NaOH and once with H_2O ; the $CHCl_3$ was discarded. The combined basic washings were acidified with 4N H_2SO_4 and then extracted 2 \times 40 ml and 2 \times 20 ml with $CHCl_3$.

The combined $CHCl_3$ fractions after either direct extraction or a basic wash step were dried with anhydrous Na_2SO_4 and then evaporated to dryness in vacuo at 20°C to minimize any losses of volatile components. The residues were dissolved in 5 ml CH_2Cl_2 -methanol (5:1), ethereal diazomethane was added, and the mixtures were allowed to stand for 30 min at room temperature. If the yellow color disappeared, additional diazomethane was added. At the end of the methylation period, the samples were evaporated to dryness in a stream of nitrogen, then redissolved in benzene and, after drying with Na_2SO_4 , made up to 5 ml in volumetric flasks.

Analyses. A 76-cm \times 2-mm i.d. glass column packed with 7% QF-1 on Chromosorb W (high performance) 100/200 mesh was used to chromatograph HCP and PCP. The injector temperature was 220°C, the column temperature was 125° or 196°C, and the detector temperature was 210°C. Under the conditions employed, hexachlorophene dimethyl ether had a retention time of approximately 6.1 min at 196°C while pentachlorophenol methyl ether showed a retention time of 1.5 min at 125°C.

Standard HCP and PCP solutions were methylated each day and aliquots then analyzed by gc. Peak areas were measured by triangulation to prepare standard curves that were linear in the range 10-200 pg HCP (as the dimethyl ether) and 20-150 pg PCP (as the methyl ether). All water and sewage samples were extracted and analyzed in duplicate. These analyses agreed within $\pm 3.2\%$ and $\pm 8.1\%$ for HCP and PCP, respectively.

Confirmation of Identity. A 5-gal sample of sewage influent estimated by gc to contain 931 μg HCP and 98.6 μg

Table 1. Recovery of Hexachlorophene and Pentachlorophenol Added to Distilled Water^a

Concentration, ppb	Amount added, ng	HCP ^b		PCP ^b	
		Amount found, ng	Percent recovery	Amount found, ng	Percent recovery
0	0	2.2, 2.7	...	ND	...
0.005	4.5	6.6, 5.9	138
0.010	9.0	10.8, 8.4	107
0.020	18	19.1, 22.5	116
0.050	45	49.5, 41.3	101	42, 43	94.4
0.100	90	88.9, 89.2	98.8	80, 85	91.6
0.200	180	190, 195	107	194	108
0.500	450	463, 463	103	580, 479	115
1.000	900	944, 938	104	975, 958	107
5.000	4500	4280, 4430	99	4549, 4639	103

^a Phenols were added to 900 ml of distilled water. After acidification and extraction, analyses were made by gas chromatography.

^b Average recoveries of HCP (except for the 0.005 ppb sample) were 104% and for PCP 103%.

PCP was collected at the grit chamber of the Corvallis sewage treatment plant. The sewage sample was acidified with H_2SO_4 and 2-liter batches were extracted three times with the same 200-ml portions of $CHCl_3$. The combined acidic extract was washed twice with 0.2N NaOH and the $CHCl_3$ discarded. The basic extracts were acidified with H_2SO_4 and the acidic material was extracted three times with $CHCl_3$. Evaporation of the combined extract to dryness in vacuo at 20°C gave 164 mg of material shown by gc analysis to contain 596 μg of HCP and 41 μg of PCP.

The entire acidic extract fractions were streaked at the origin of two 20-cm² sheets of Mallinckrodt Chrom AR 1000 preparative thin-layer chromatography (tlc) support, and the sheets then developed in benzene-acetic acid-water (2:1:1). A uv absorbing band ($R_f = 0.83$), having the same mobilities as the HCP and PCP standards, was cut out and eluted with $CHCl_3$ to yield 51 mg of purified material. This fraction was streaked at the origin of a 20-cm² preparative Chrom AR 1000 sheet developed in benzene-butanol-ammonia (2:5:2). A uv absorbing band ($R_f = 0.89$) migrating with the HCP standard was cut from the plate and eluted to give 5 mg of material that, by gc analysis, contained 130 μg HCP. A small uv absorbing zone ($R_f = 0.50$), having the same mobility as authentic PCP, was also eluted to yield 10 mg of material that, according to gc analysis, contained 30 μg PCP.

The HCP fraction was further purified by tlc on a 10 \times 20-cm plate made from silica gel GF (Brinkmann Instrument Co., Great Neck, N.Y.) and developed in butanol-benzene-ammonia (1:1:1). Elution of the large uv absorbing zone ($R_f = 0.49$) which moved with the standard HCP, gave a purified fraction suitable for mass spectrometry.

Similarly, the crude PCP fraction was streaked at the origin of a 20-cm² silica gel GF plate which was then developed in butanol-benzene-ammonia (1:1:1). The small uv absorbing zone at $R_f = 0.18$ was scraped from the plate and eluted to give a product that was utilized for mass spectrographic analysis.

The HCP and PCP in sewage effluent were extracted and purified in a similar manner except that initial tlc occurred on a 20-cm² silica gel GF plate developed in benzene-acetic acid-water (2:1:1) followed by subsequent cleanup on a silica gel GF plate developed in benzene-butanol-ammonia (1:1:1). Purified fractions containing HCP and PCP were obtained in this manner.

Willamette River water (111 liters), shown by gc analy-

sis to contain 2.21 μg HCP and 27.6 μg PCP, was acidified and extracted in 1.5-liter batches with $3 \times 100\text{-ml}$ CHCl_3 to give a combined acidic extract weighing 7.1 mg and containing 1.97 μg of HCP and 20.1 μg of PCP. This material was streaked at the origin of a single 20-cm² silica gel GF plate. The plate was developed in benzene-butanol-ammonia (1:1:1). Fractions containing the purified PCP (uv absorbing zone $R_f = 0.25$) and HCP (no uv absorption, located by standard $R_f = 0.53$) were eluted from the plate and used for subsequent mass analysis.

In addition, effluent water from the Corvallis Taylor water plant, which takes raw water from the Willamette River, was also extracted to recover HCP and PCP. A total of 135 liters of treated water was acidified and subjected to extraction with CHCl_3 in 1.5-liter portions. Purified HCP and PCP were then isolated for mass spectrometry after tlc cleanup of the acidic extract.

Results and Discussion

At least 90-95% recoveries of radioactivity were obtained in sewage and river samples spiked with 5 ppb methylene-¹⁴C-hexachlorophene, indicating that no significant losses of HCP occurred during the normal or basic extraction procedure. Recoveries of HCP and PCP added to distilled water at concentrations ranging between 0.005-5.0 ppb averaged 104 and 103%, respectively, when the two chlorophenols were analyzed by gc (Table I). Recoveries were comparable whether the water samples were acidified and extracted immediately or after standing for 2 hr in Pyrex containers, indicating that no appreciable binding of the phenols to glass occurred under these conditions. Analysis of river water and sewage influent by gc, using the method of additions, also gave good recoveries, ranging between 78-103% for HCP and 85-104% for PCP in river water (0.2-10 ppb chlorophenol added) and 94-114% for HCP (12.5-100 ppb added) and 89-102% for PCP in sewage influent (1-10 ppb added).

The hourly concentrations of HCP and PCP entering the Corvallis sewage treatment plant on September 10, 1969, are shown in Figure 1. Although the levels fluctuated considerably, maximum influent concentrations of HCP were attained at about 8-9 a.m. and again at 8 p.m. Since the average transit time from the point of discharge

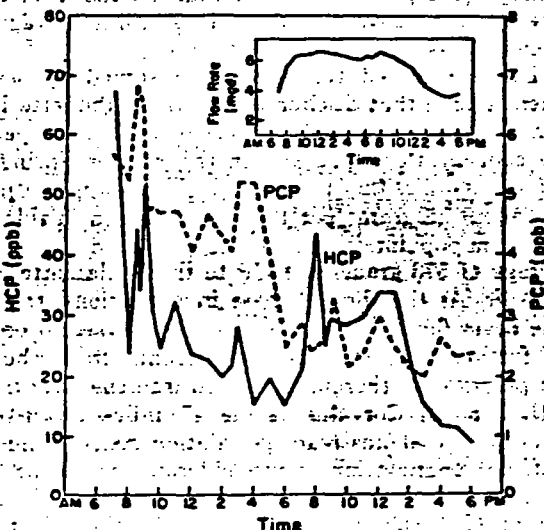


Figure 1. Hourly concentrations of hexachlorophene and pentachlorophenol in the Corvallis, Ore., sewage influent on September 10, 1969

Inset—Influent flow rates in million gallons per day, mgd

Table II. Concentration and Percent Removal of Hexachlorophene and Pentachlorophenol by the Corvallis, Ore., Sewage Treatment Plant for Seven Consecutive Days^a

Day	HCP, g/24 hr ^b			PCP, g/24 hr ^b		
	Influent	Effluent	Percent removal	Influent	Effluent	Percent removal
Wednesday	707	175	75.3	109.0	51.9	52.4
Thursday	604	163	73.0	79.3	38.4	51.6
Friday	578	142	75.4	70.1	21.7	69.0
Saturday	542	129	76.3	82.0	32.8	60.0
Sunday	366	92.2	74.8	64.2	24.5	61.8
Monday	422	110	74.0	83.4	33.3	60.1
Tuesday	450	157	65.1	68.9	71.1 ^c	0
		Mean	73.4			59.0

^a Samples collected from September 24-30, 1969.

^b Mean of duplicate determinations.

^c Possible contamination of sample.

to the Corvallis plant is about 2 hr, greatest entry of HCP into the sewage lines occurred between 6-7 a.m. and at 6 p.m. These periods appear to correspond to the most likely time of the day when maximum home use of germicidal soaps, deodorants, and other personal care products containing HCP would be expected to occur.

Highest intake of PCP into the sewage treatment plant took place during the daytime period reflecting probable industrial discharge of this chlorophenol. Minimum HCP and PCP levels were attained from 2-6 a.m., during the period of minimum sewage flow and activity of the population.

Analyses of a composite sample for the same 24-hr period obtained from an automatic sampling device at the Corvallis plant gave mean HCP and PCP concentrations of 22.1 and 4.3 ppb, respectively. When one takes into account the average sewage flow for that day (5.75 million gal), these concentrations corresponded to a daily discharge from the city of Corvallis of about 482 grams of HCP and 93.6 grams of PCP.

Suspended material was removed from an aliquot of the sewage influent by centrifugation. Gc analysis of this material and the supernatant fraction showed that about 50% of the HCP in the influent was associated with the sediment and 50% was in solution. Most of the PCP, on the other hand, was in solution. Strong binding of HCP to proteins has been previously reported (Flores and Buhler, 1972).

To examine the daily fluctuations in total HCP and PCP input into a typical sewage treatment facility and the efficiency of removal of the chlorinated phenols by the plant, daily composite influent and effluent samples were collected over a seven-day period from the Corvallis plant. Analyses of these samples showed that daily HCP intake ranged from 366-707 grams with the maximum occurring on Wednesday, September 24, 1969 (Table II). Maximum quantities of PCP also appeared in the sewage influent on the same date. After the midweek maximum, HCP and PCP concentrations gradually decreased to the lowest levels on Sunday and then gradually increased on Monday and Tuesday. About 73% of the HCP and 59% of the PCP entering the plant was removed during the treatment process.

To help determine the most efficient stage for the removal of chlorophenols from sewage, fractions were collected at various steps during the secondary treatment process in the Corvallis plant and analyzed for HCP and PCP (Table III). HCP to 71% and 44% of the PCP were

Table III. Concentration and Percent Removal of Hexachlorophene and Pentachlorophenol at Various Stages Within the Corvallis, Ore., Sewage Treatment Plant^a

Stage sampled in plant	Time sampled ^b	Concentration, ppb		Percent removal	
		HCP	PCP	HCP	PCP
After grit chamber (influent)	9:00	22.2	0.91
After primary clarifier	10:00	21.6	1.10	2.7	0.0
After trickling filter	10:30	6.4	0.51	71.4	44.0
After chlorination	11:00	6.8	0.49	69.4	46.2
Out to river (effluent)	13:00	7.3	0.33	67.1	63.7

^a Samples collected on September 24, 1969.

^b Sampled at the times indicated to compensate for flow times between the various stages of operation.

^c Mean of duplicate extractions and analyses.

Table IV. Levels of Hexachlorophene and Pentachlorophenol in 24-Hr Composite Influent and Effluent Samples and Calculated Total Output from Corvallis, Eugene, and Salem, Ore., Sewage Treatment Plants^a

Chlorophenol	Cities	Influent, ppb ^b		Percent removal	Output, g/10,000 pop., 24 hr	
		Influent	Effluent		Output	g/10,000 pop., 24 hr
HCP	Corvallis ^c	30.8	12.2	60.4	245	66.5
	Eugene ^d	20.0	5.6	72.1	244	44.0
	Salem ^e	30.0	12.1	59.7	973	122.0
PCP	Corvallis ^c	1.4	1.0	28.6	20	5.5
	Eugene ^d	4.1	3.3	19.5	144	26.0
	Salem ^e	4.6	4.4	4.4	267	33.6

^a Collected simultaneously on November 13, 1969.

^b Mean of duplicate extractions and analyses.

^c Corvallis plant serves an approximate population of 36,800. Influent flow 5.3 million gal/day on the date sampled.

^d Eugene plant serves an approximate population of 60,000. Influent flow 12.5 million gal/day on the date sampled.

^e Salem plant serves an approximate population of 80,000. Influent flow 21.25 million gal/day on the date sampled.

removed by the trickling filter suggesting that these compounds were subject to considerable biological degradation.

In addition, some chlorophenol was also adsorbed onto digested sludge and ultimately removed for use as land fill. Analyses of sludge samples by gc showed that they contained about 6.7 ppb HCP on a wet basis or 137 ppb HCP in a dried sample. By contrast, no detectable amounts of PCP were found associated with the sewage sludge.

It was of interest to compare HCP and PCP levels entering and leaving different sewage treatment plants to establish whether the results from the Corvallis plant were typical. Accordingly, 24-hr composite influent and effluent samples were collected simultaneously from the Corvallis, Eugene, and Salem sewage treatment plants. These three plants have secondary sewage treatment processes that serve populations of 36,800, 60,000 and 80,000 people, respectively, and all discharge their effluents into the Willamette River. Eugene is located 47 miles upstream and Salem 48 miles downstream from the city of Corvallis.

HCP concentrations in the influents were similar for the three facilities (Table IV), but when flow was taken into account, the input of HCP into the Salem plant was considerably higher. The highest percentage (72%) of HCP was removed by the Eugene plant while the Salem plant

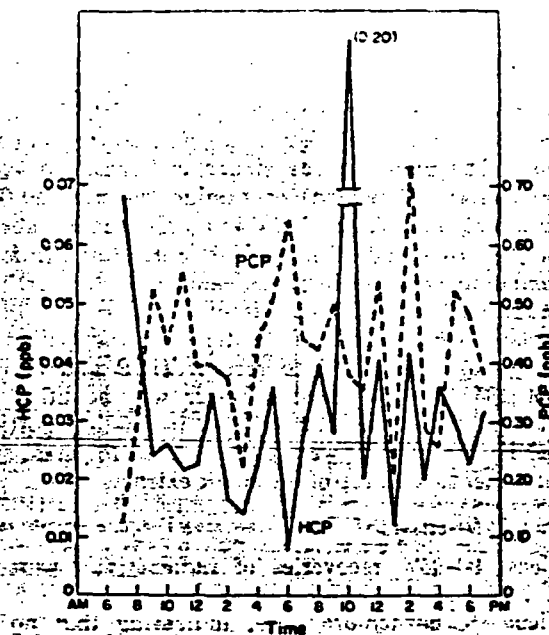


Figure 2. Hourly concentrations of hexachlorophene and pentachlorophenol in the Willamette River on September 30, 1969

was the least efficient (60%). Release of HCP into the Willamette River was greatest from the Salem facility even when calculated on a basis of per 10,000 population served.

The influent concentration and total daily intake of PCP were also highest in the Salem plant (Table IV). Most of the PCP entering the plant on this date, however, was apparently not removed but instead was discharged into the river. Total daily releases of PCP varied between 20 and 267 grams from the three plants, with Salem showing the highest discharge on a 10,000-population basis.

One factor that may contribute to the differences in removing the chlorophenols by the three sewage treatment plants is the variable composition of the influent sewage. Chemical composition of the sewage influent varies with such items as the time of day, the sources of industrial wastes, and the season of the year and could influence the treatment efficiency of the plant. These differences in composition were reflected in marked variations in the chromatographic patterns observed among the three cities or between successive analyses of Corvallis sewage influent and effluent in the present study.

We have estimated the population served by sewage treatment plants that discharge into the Willamette River upstream from the city of Corvallis to be 104,000 people, mainly concentrated upstream in the Eugene-Springfield area. If one assumes that the average 24-hr sewage output of HCP is about 55 grams per 10,000 population (Table IV), the upstream population can then be estimated to discharge an average of 573 grams of HCP to the Willamette River every 24 hr. At the time of these investigations, the Willamette River flow at Corvallis was 5800 ft³/sec. If one assumes further that the sewage effluents from the upstream plants were thoroughly mixed into the river by the time they reach Corvallis (some 47 miles downstream from the principal upstream population center) and that insignificant degradation of this chlorophenol occurs during its passage to Corvallis, one could then calculate the theoretical HCP content of the river to be about 0.04 ppb. Using similar assumptions, one can estimate the concentration of PCP in the Willamette River to be only 0.01 ppb.

Levels of HCP and PCP present in the Willamette

River at Corvallis were analyzed hourly over a 24-hr period, and the results are shown in Figure 2. Levels of HCP ranged between 0.01 and 0.2 ppb, with most values falling in the range of 0.01–0.04 ppb. HCP is apparently fairly resistant to degradation in the stream since the average concentration of the chlorophenol in the water during this time period was 0.04 ppb, in excellent agreement with the estimated concentration at release. The unusually high HCP concentration in the 10 p.m. sample is difficult to understand since high levels were in both of the duplicate water samples, tending to rule out the possibility of contamination during the extraction or analysis.

During the same time period, the concentrations of PCP in the Willamette River fell between 0.10 and 0.70 ppb (Figure 2). These values were at least tenfold higher than the estimated value of 0.01 ppb, suggesting that either our assumptions were wrong or that additional PCP was entering the Willamette River from sources other than municipal sewage. In this regard, Rudling (1970) found PCP levels as high as 9 ppb in water immediately downstream from a pulp mill releasing PCP in its effluent. A number of paper mills, plywood mills and other industries in the Eugene-Springfield area that discharge their waste effluent directly into the Willamette River may thus provide additional sources of PCP input to the river. The mean PCP concentration in the Willamette River samples analyzed during this period was 0.41 ppb. On a basis of the 5800 ft³/sec river flow on that date, the total PCP content in the Willamette River water passing Corvallis during this 24-hr period was calculated to be about 5.82 kg.

The city of Corvallis obtains its drinking water, in part, by treatment of Willamette River water. To determine whether significant amounts of these chlorophenols were still present in the finished water, the concentrations of HCP and PCP were analyzed at various stages of the treatment process. Analysis of an incoming raw river water sample showed that it contained 0.03 ppb HCP and 0.17 ppb PCP, respectively (Table V). One third of the HCP was removed during the Al₂(SO₄)₃ flocculation indicating that an appreciable association of the HCP occurred with particulate material. Nevertheless, about one third of the HCP originally present in the river water remained in the effluent from the water treatment process. PCP was also extensively removed from the incoming water during the flocculation step, but the finished water still contained 0.06 ppb PCP (Table V).

Table V. Concentration and Percent Removal of Hexachlorophene and Pentachlorophenol at Various Stages of the Water Treatment Process in Taylor Water Treatment Plant for City of Corvallis, Ore.^a

Stage sampled in plant	Time sampled, a.m. ^b	Concentration, ppb ^c		Percent removal	
		HCP	PCP	HCP	PCP
Raw Willamette River water (influent)	8:00	0.03	0.17
At flocculation step	8:27	0.02	0.06	33	65
After sedimentation	10:05	0.02	0.04	33	76
After filtration (final product)	10:15	0.01	0.06	67	65

^a Samples collected on May 26, 1970.

^b Sampling times were calculated on the basis of known volumes, delay times, and a flow of 6700 gal/min of water pumped into the treatment plant.

^c Mean of duplicate extractions and analyses.

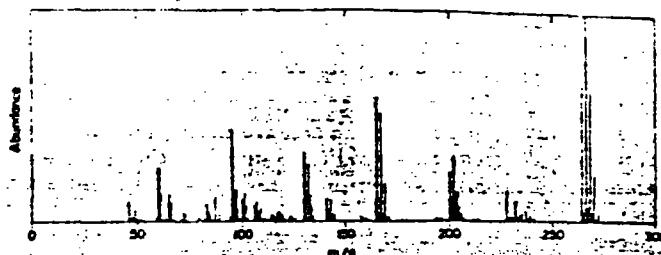


Figure 3. Mass spectrum of hexachlorophene, C₁₂H₆O₂Cl₆.

Major peak assignments m/e: 404, molecular ion; 369, loss of Cl; 334, loss of 2 Cl; 299, loss of 3 Cl; 270, loss of 3 Cl and CHO; 241, loss of 3 Cl and 2 CHO; 236, loss of 4 Cl and CO; 209, loss of C₆H₂OCl₅; 208, loss of C₆H₃OCl₅; 196, loss of C₇H₂OCl₅; 180, loss of C₈H₂OCl₅ and CO; 173, loss of C₆H₂OCl₅ and Cl; and 145, loss of C₆H₂OCl₅, Cl, and CO.

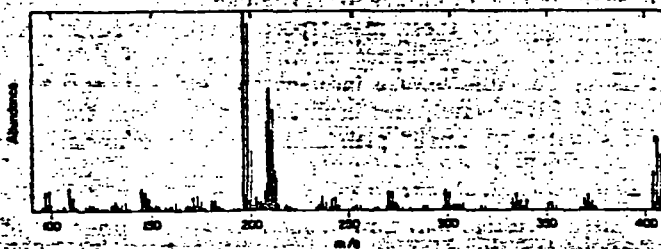


Figure 4. Mass spectrum of pentachlorophenol, C₆HCl₅O.

Major peak assignments m/e: 264, molecular ion; 235, loss of CHO; 228, loss of Cl; 201, loss of Cl and CHO; 200, loss of Cl and CO; 185, loss of 2 Cl and CO; and 130, loss of 3 Cl and CO.

The results of the present investigation were based on gc analyses of HCP and PCP. Although the water and sewage extracts contained peaks with the correct retention times for methylated HCP and PCP and addition of the authentic compounds to extracts gave single symmetrical peaks, it is possible that other components of water or sewage, not related to HCP or PCP, might have identical gc retention times and, thus, interfere with the analyses. Chromatography of unmethylated water and sewage extracts, however, did not yield any gc peaks in the region where methylated HCP and PCP are eluted. Moreover, it is highly unlikely that there would be one or more acidic components in water or sewage that could be methylated to yield products with the same retention times as methylated HCP or PCP. In addition, aliquots of the sewage influent and effluent extracts after methylation were also subjected to chromatography in a gas chromatograph equipped with a microcoulometric detector, and the presence of halogen was verified in the case of both peaks.

Final verification of the identity of HCP and PCP in water and sewage, however, was obtained by mass spectrometry after purification of appropriate extracts by preparative tlc. Material purified in this manner was introduced into the probe assembly of the mass spectrometer and heated to 75°C for identification of HCP. The mass spectra of the HCP fractions from sewage influent and effluent, river water, and treated drinking water were identical to the spectrum for authentic hexachlorophene (Figure 3). This spectrum is characterized by a parent ion peak at m/e of 404 (19.7%) exhibiting a characteristic 6 chlorine pattern (Beynon et al., 1968) and major 3 chlorine patterns at m/e of 209 (61.5%), 208 (44.6%), and 196 (100%). Methylation of the various HCP isolates yielded material with the mass spectral characteristics of HCP dimethyl ether with a 6-chlorine parent ion peak at m/e 432 (9.3%) and major 3-chlorine fragments at m/e 209

(35.2%) and at m/e 223 (52.3%). In addition, the total acidic extract from a sewage effluent sample, after a basic wash procedure, was methylated and an aliquot injected into a Varian Model 1400 gc (equipped with a 4 × 1/8-in. Pyrex column containing 7% DC-11 on 100/120 High Performance W operated at 250°C) coupled to the mass spectrometer. The mass spectrum of the HCP dimethyl ether peak was identical to that of the authentic methylated phenol.

Similarly, mass analyses of PCP extracts from sewage, river water, and treated drinking water heated to 25°C in the probe assembly yielded spectra identical to that for the authentic PCP (Figure 4). These spectra contained a 5-chlorine parent ion peak at m/e 264 (65.8%) plus a large number of typical multichlorine peaks representing fragments formed by loss of Cl, CO, or CHO. Rudling (1970) has also employed mass spectrometry to establish the presence of PCP in surface water samples.

The results of these mass spectrographic studies have confirmed the gc evidence for the presence of HCP and PCP in Corvallis sewage influent and effluent, raw Willamette River water, and in finished water from the Corvallis water treatment plant. The isolated materials exhibited tlc properties and mass spectra that were identical to those for authentic HCP and PCP reference standards.

These investigations have demonstrated the presence of significant concentrations of HCP and PCP in municipal sewage, river water, and treated river water. Concentrations of these chlorophenols presently occurring in Oregon

sewage and water are not known but recent U.S. Food and Drug Administration action to restrict the use of HCP may have decreased the amounts of this chlorophenol reaching the aquatic environment.

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Toxaphene Accumulation in Fish in Lakes Treated for Rough Fish Control

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In the U.S., many northern, temperate zone, seepage, and drainage lakes are shallow, homothermal, nutrient-rich bodies of water. As such, they can pose a serious problem to those responsible for lake management. These lakes usually support productive fisheries of high recreational value, but maintaining the delicate balance between certain rough fish (buffalo, bullheads, and carp) or stunted panfish and game fish is difficult. In general, attempts to remove unwanted fish by physical methods have not been successful because complete removal is precluded. Although partial removal may bring improvement, this condition deteriorates owing to the prolific nature of rough fish. In the attempt to overcome fish population deficiencies that have come about with and without man's interference, the practice of eradicating fish in a lake by means of a chemical toxicant, such as toxaphene, and restocking with wanted species has often been used. In Wisconsin this practice provided opportunities to study the decline of toxaphene concentrations in treated lakes, to monitor the extent of toxaphene residue uptake by stocked fish, and to compare the toxicity of "aged" toxaphene residues to that of the standard mixture. Results of such a study are presented.

Toxaphene Characteristics

General Characteristics. Toxaphene, an insecticidal chemical referred to as chlorinated camphene, is prepared by agitating one part commercial camphene dissolved in five parts of carbon tetrachloride and chlorinating with chlorine gas under ultraviolet light for 30–35 hr until a product with a chlorine content of 67–69% is obtained (Buntin, 1951). The preparation results in a complex, multicomponent mixture with an empirical formula $C_{10}H_{10}Cl_8$. Various values ranging from 0.4 mg/l. (Cohen et al., 1960) to 3 mg/l. (Hercules, 1962) have been reported for water solubility of toxaphene. However, these values are probably high since Hughes (1968) determined by extraction and gas chromatographic (gc) analysis that toxaphene concentrations in filtered lake water never exceeded 37 µg/l. even when the water was sampled shortly after application of a calculated 0.15 mg/l. dose.

Toxaphene Toxicity. Reported values of the toxicity of toxaphene to fish vary, probably because fish react differently in waters of different chemical composition and temperature. By any estimation, however, toxaphene must be classed as an extremely potent poison for fish. Henderson et al. (1959) lists its toxicity as second only to endrin among the common chlorinated pesticides. Several investigators have observed the gross effects of toxaphene poisoning on fish (Tanner and Hayes, 1955; Ludemann and Neumann, 1960; Workman and Neuhold, 1963). Symptoms are fast, erratic swimming, occasional bump-

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